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Highly sensitive C-reactive protein levels in Iranian patients with pulmonary complication of sulfur mustard poisoning and its correlation with severity of airway diseases

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Abstract

Background: Sulfur mustard (SM) is a chemical warfare agent that can cause serious pulmonary complications. This study was designed to determine serum highly sensitive C-reactive protein (hs-CRP) and evaluate its correlation with lung function parameters in patients with chronic obstructive pulmonary disease (COPD) due to SM poisoning. **Methods:** Fifty consecutive SM patients with stable COPD and a mean age 46.3 $\pm$ 9.18 years were enrolled in this cross sectional study. Thirty healthy men were selected as controls. Lung function parameters were evaluated. Serum hs-CRP by immunoturbidometry assay was measured in both the patients and controls. **Results:** In the case group, the mean forced expiratory volume in one second (FEV1) was 2.14 $\pm$ 0.76 L (58.98% $\pm$ 17.51% predicted). The mean serum hs-CRP was 9.4 $\pm$ 6.78 SD and 3.9 $\pm$ 1.92 SD mg/L in the cases and controls, respectively, with significant statistical differences ($p < .001$). There was negative correlation between the serum hs-CRP and FEV1 levels ($p = .01$). The serum hs-CRP levels were also correlated with Global Initiative for Chronic Obstructive Lung disease (GOLD) stages ($r = .45$, $p < .001$). **Conclusions:** Our findings suggest that the serum hs-CRP level is increased in SM patients with COPD and may have a direct correlation with disease severity. It may then be used as a marker for the severity of COPD in patients with SM poisoning.

Keywords

chemical warfare agents, sulfur mustard gas, veterans, chronic obstructive pulmonary disease, highly sensitive C-reactive protein

Introduction

Sulfur mustard (SM) is a chemical warfare agent that was used in large scale during the World War I and in the Iran–Iraq conflict between 1983 and 1988.[1] During the Iran–Iraq war, over 100,000 Iranians were exposed to sulfur mustard, of which 45,000 patients are now suffering from complications of SM poisoning.[2] SM is a potent, toxic, alkylating agent that is able to cause serious ocular, neurologic, cutaneous, bone marrow, immunodeficiency, and pulmonary complications.[1]

Respiratory problems are the most common late complications of SM poisoning in the veterans.[3-5] Ghanei et al. reported that chronic bronchitis is the most frequent late respiratory disease among Iranians exposed to mustard gas.[6] A unique form of chronic obstructive pulmonary disease (COPD), named

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"Mustard Lung," was proposed in patients with late respiratory complications of SM poisoning.[3]

As COPD is a systemic inflammatory condition, inflammatory markers such as C-reactive protein (CRP), interleukin 6, and tumor necrosis factor α (TNF-α) have important roles in its pathogenesis. Highly sensitive C-reactive protein (hs-CRP) is one of the important inflammatory markers and is mainly synthesized by hepatocytes during inflammatory reactions.[7] In the last decade, concern has grown about significant correlations of hs-CRP with myocardial infarction, unstable angina, and stroke.[8] It is now believed that hs-CRP levels also relate to the presence of airflow obstruction.[7,8] Pinto-Plata et al.[8] and Ridker[9] found that hs-CRP levels are elevated in COPD patients without clinically relevant ischemic heart disease, independent of cigarette smoking. The prevalence of increased hs-CRP in COPD patients has been examined in the National Health and Nutrition Examination Survey III, where 41% of patients with moderate COPD had hs-CRP levels >3 mg/L, and 6% had levels >10 mg/L. In comparison, 52% of patients with severe COPD had hs-CRP levels >3 mg/L and 23% had levels >10 mg/L.[10,11] Dahl et al.[12] suggested that hs-CRP is a strong and independent predictor of future COPD outcomes in individuals with airway obstruction. Hs-CRP is higher in patients with low forced expiratory volume in one second (FEV1) and in smokers.[13] In addition, hs-CRP is elevated in stable and exacerbated states of COPD.[14,15] It is possible that elevated levels of hs-CRP are also a predictor of cardiovascular disease in COPD patients.[16]

The present study is the first, of which we are aware, that was designed to determine serum hs-CRP and evaluate its correlation with lung function parameters in patients with COPD due to SM poisoning.

Materials and methods

Subjects

The current cross-sectional study was performed on the chemical war gassed veterans with stable COPD aged 37 to 65 years attending the pulmonary clinic of Ghaem hospital, in Mashhad, Iran between April and September 2008. All patients had validated documents of SM gas exposure and suffered from complications of SM poisoning. The time since SM exposure ranged from 19 to 23 years. All studied subjects were non-smoker males. Patients with all levels of severity of COPD were included in the study if they had a post-bronchodilator FEV1/FVC (forced vital capacity) < 0.7 after 400 μg of inhaled albuterol, according to the definition of the American Thoracic Society (ATS).[17] The patients were excluded if their FEV1 increased more than 12% and 200 mL after bronchodilator; or those who had asthma, bronchiectasis, tuberculosis, active pulmonary infections, cardiovascular diseases, cerebrovascular diseases, diabetes mellitus, or other confounding inflammatory diseases, such as malignancy, arthritis, connective tissue diseases, and inflammatory bowel disease or if they had exacerbation of disease or hospitalization during the last 2 months. Also those patients who had taken aspirin and statins were excluded. Thirty healthy non-smoker men with no history of pulmonary, cardiovascular, or inflammatory disease were enrolled as a control group for hs-CRP analysis. Their age and weight were matched with cases.

Interview and physical examination

All subjects were visited by one chest physician. In addition to a detailed physical examination, a questionnaire was completed by physician for each patient that included the following items: age, duration of disease, date of sulfur mustard exposure, current inhaled corticosteroid therapy, and home oxygen therapy. Weight (with clothes on) and height (without shoes) were measured for each participant, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. All subjects completed the St George's Respiratory questionnaire (SGRQ) on health impairment; while sited at a desk in a quiet area free from distraction.[18] Using the SGRQ, the symptoms, activity, impact, and total scores were calculated for each patient with an Excel-based scoring calculator.[18]

Lung function tests

Forced expiratory volume in one second, FVC, and FEV1/FVC were measured by standard spirometric techniques (Multi-Functional Spirometer HI-801, Chest M.I., Inc, Tokyo, Japan) and the values were expressed as a percentage of the predicted values. The best of three consecutive recordings of spirometry was used. The severity of COPD was assessed using the Global Initiative for Chronic Obstructive Lung disease (GOLD) guidelines.[19] Dyspnea was assessed by the Modified Medical Research Council (MMRC) scale,[20] which was graded from 0 to 4 scales according to the patient's complaint. Patients performed the 6-min walk test (6MWT)

Table 1. Clinical and pathophysiological parameters of 50 patients with respiratory complications of sulfur mustard poisoning

Clinical and pathophysiological parameters	Values
Age (yrs)*	46.3 ± 9.18
Duration of disease (yrs)*	17.00 ± 6.00
FEV1 (L)*	2.14 ± 0.76
FEV1 (% Pred.)*	58.98 ± 17.51
FEV1/FVC*	62.14 ± 9.70
GOLD stage (% of patients)	
I	7(14)
II	27(54)
III	12(24)
IV	4(8)
6MWD (m)*	327.8 ± 86.96
BMI*	27.32 ± 3.30
PaO_2 (mm Hg)*	75.1 ± 12.00
SaO_2 (%)*	94.03 ± 3.45
$PaCO_2$(mm Hg)*	55.37 ± 9.50
Total St George Score*	56.23 ± 14.31
Inhaled corticosteroid therapy (% of patients)	21(42)
hs-CRP (mg/l)*	9.4 ± 6.78

*The data are presented as the mean ± SD. FEV1, forced expiratory volume in one second; FVC: forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease; 6MWD: 6-min walk distance; BMI: body mass index; PaO_2: arterial oxygen tension; S_aO_2: arterial O_2 saturation; $PaCO_2$: arterial carbon dioxide tension; SGRQ: Saint George's Respiratory Questionnaire; hs-CRP: highly sensitive C-reactive protein.

according to the ATS guidelines[21] in a 30-m flat and straight indoor corridor with marking every 3 m. The body mass index, obstruction, dyspnea, and exercise capacity (BODE) index was calculated in all patients with total possible values range from 0 to 10.[20]

Blood samples

Blood samples were obtained in non-fasting conditions and at rest. Serum hs-CRP was measured by immunoturbidometry assay (Roche Diagnostics, Mannheim, Germany) and an auto analyzer (Lysis, Milan, Italy), with a normal value defined as less than 5 mg/L. The maximum interassay and intra-assay coefficient of variation for the range of hs-CRP concentrations evaluated were 3.5%. The lower limit of detection was 0.1 mg/L. The samples were identified by bar code, so that samples from controls and COPD patients were randomly distributed among the assay plates. Arterial blood gases were measured by a blood gas analyzer (AVL 995; AVL Scientific Corporation, Roswell, GA., USA). The study was approved by the ethics committee of the Mashhad University of Medical Sciences (MUMS). All patients gave informed consent.

Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS, version 14.0). The variables are presented as percentages and means ± SDs. Descriptive statistics were used to summarize the demographic characteristics of the case and control groups. The normality of continuous variables was checked using the one sample Kolmogorov-Smirnov test. For continuous and categorical variables, independent student's t tests and chi-square tests were used to evaluate the statistical significance of any difference or relationship between parameters, respectively. Pearson and Spearman correlation coefficients were calculated. p Values less than .05 were considered significant.

Results

A total of 50 consecutive male patients were included in this study. The mean age of the patients was 46.3 ± 9.18 years with BMI of 27.32 ± 3.30 kg/m^2. The controls were matched by age and BMI to the patients (47.8 ± 7.9 years and 27.2 ± 2.1, respectively).

The majority of patients were in GOLD stages II and III (54% and 24%, respectively). Twenty-one patients (42%) were taking inhaled corticosteroid and 9 (18%) were on home oxygen therapy. The demographic and clinical characteristics of the patients are summarized in Table 1. The mean serum hs-CRP levels were significantly higher in the patients than in the controls (9.4 ± 6.78 SD versus 3.9 ±1.92 SD mg/L, $p < .001$). Out of 50 patients in the case group, 32 (64%) had high serum hs-CRP levels (≥5 mg/L).

The correlations of hs-CRP with the potential parameters of COPD severity in the patients were examined and are shown in Table 2. There was a correlation between the GOLD stages and hs-CRP ($r = .45$, $p = .001$) as illustrated in Figure 1. Also, other correlations were found between hs-CRP and the following parameters: FEV1 ($r = –.32$, $p = .01$; Figure 2), FEV1 percent predicted ($r = –.36$, $p = .010$), and $PaCO_2$ ($r = .37$, $p = .009$).

As shown in Table 3, higher GOLD stages had higher frequencies of patients with elevated hs-CRP levels ($p = .041$). The mean hs-CRP levels were not statistically different between patients on inhaled

Table 2. Correlation of hs-CRP with other parameters in 50 patients with respiratory complications of sulfur mustard poisoning

Variables	Correlation coefficient (*r*)	*p*-Value
GOLD stage	.45	.001
BMI	.27	.056
Duration of disease	.082	.573
FEV 1	–.32	.010
FEV 1 % Predicted	–.36	.010
P_aCO_2	.37	.009
P_aO_2	–.06	.695
MMRC	–.14	.322
6 MWD	–.14	.330
BODE index	.07	.613
Total SGRQ score	–.2	.85

Hs-CRP, highly sensitive C-reactive protein; GOLD, Global Initiative for Chronic Obstructive Lung Disease; BMI, body mass index; FEV1, forced expiratory volume in one second; $PaCO_2$, arterial carbon dioxide tension; PaO2, arterial oxygen tension; MMRC, Modified Medical Research Council; 6MWD, 6-min walk distance; BODE, body mass index, obstruction, dyspnea and exercise capacity, SGRQ, ST George Respiratory Questionnaire.

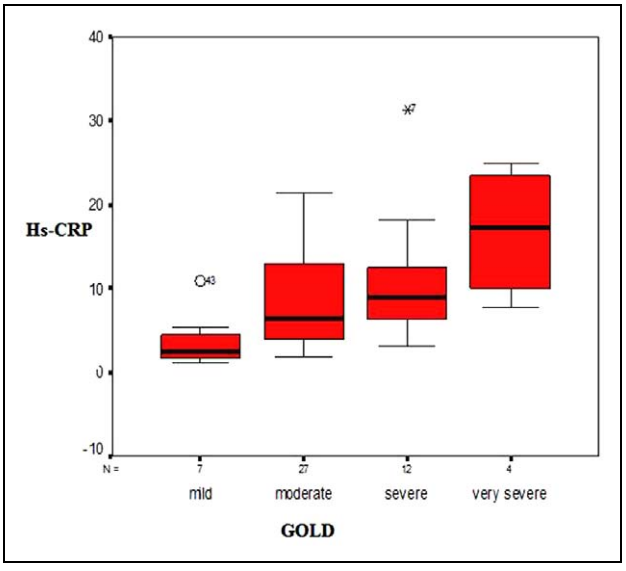

Figure 1. Correlation between highly sensitive C-reactive protein (hs-CRP) and global initiative for chronic obstructive lung disease (GOLD).

steroid therapy and those who were not (9.1 ± 7.0 versus 8.6 ± 6.9, p=0.64). Additionally, there was negative correlation between BMI and the duration of disease ($r = –.28$, $p = .04$).

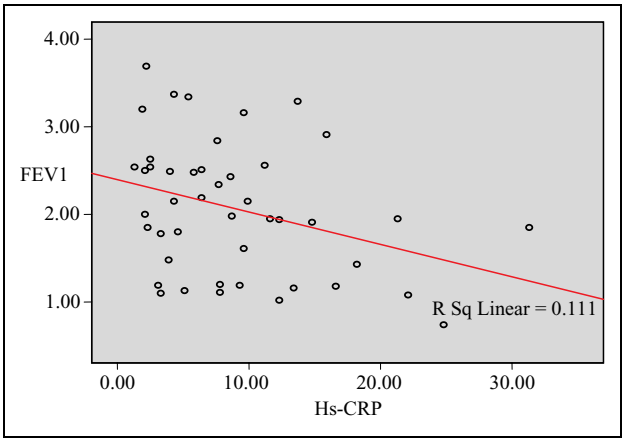

Figure 2. Correlation between forced expiratory volume (FEV) and highly sensitive C-reactive protein (hs-CRP).

Table 3. The relation between GOLD stages and serum hs-CRP in 50 patients with respiratory complications of sulfur mustard poisoning

GOLD	Low hs-CRP (<5mg/L)	High hs-CRP (≥5 mg/L)	Total
1	5 (71.4%)	2 (28.6%)	7 (100%)
2	11 (40.7%)	16 (59.3%)	27 (100%)
3	2 (16.7%)	10 (83.3%)	12 (100%)
4	0 (0.0%)	4 (100.0%)	4 (100.0%)
Total	18 (36.0%)	32 (64.0%)	50 (100.0%)

GOLD, Global Initiative for Chronic Obstructive Lung Disease; hs-CRP, highly sensitive C-reactive protein.

Discussion

To our knowledge, the present study was the first to evaluate the relationship between hs-CRP as an inflammatory marker and clinical and paraclinical severity factors in patients with respiratory complication of SM poisoning. The important findings of this study were significant correlations of hs-CRP with the GOLD stages, FEV1, and $PaCO_2$ in these patients.

In this study, we found that, despite the exclusion of smoking, cardiovascular diseases, infections, and other important inflammatory conditions, hs-CRP levels are raised in SM patients and that significant differences in the mean hs-CRP levels exist between patients and controls. These findings are similar to those of de Torres and colleagues[7] in COPD patients. In addition to COPD, which is the most frequent clinical pulmonary complication of SM exposure,[3] a recent pathologic study of surgical lung biopsies revealed that bronchiolar disease is the main pathologic finding in this group of patients.[22] This study[22]

showed that obliterative bronchiolitis (or features that strongly suggest its presence) and varying degrees of bronchiolar inflammation are the most common bronchiolar pathologic findings in this population. Our finding that 64% of patients had high hs-CRP levels, taken together with this previous research, implies that inflammation plays an important role in patients with respiratory complication of SM poisoning, especially in more advanced stages.

In the present study, the hs-CRP levels were inversely correlated with FEV1 and FEV1 percent predicted and directly correlated with the GOLD stage and P_aCO_2. In the de Torres et al. study of stable COPD patients,[7] the hs-CRP levels were mainly correlated with physiological parameters, such as FEV1, FVC, inspiratory capacity (IC) to total lung capacity (TLC) IC/TLC, 6MWT, PaO_2, and BMI, and other factors such as the BODE index and GOLD stages. Our results demonstrated that, as in other COPD patients, the hs-CRP levels increased with progression of COPD severity in SM patients. Hs-CRP may have an important role as an inflammatory marker of disease severity in mustard lung patients as it does in other COPD patients. Gan and colleagues[13,14] were the first to note the importance of high CRP levels in COPD patients who actively smoked, had reduced lung function, or had stable COPD. It has also been reported that COPD patients have higher levels of CRP independent of cardiovascular risk factors.[7,8] Broekhuizen et al.[23] found that hs-CRP was a marker of impaired energy metabolism, functional capacity, and distress in 102 severe COPD patients. Dahl et al.[12] have proposed that plasma CRP may not only be useful in assessing inflammation during the course of COPD but may also be a marker for monitoring inflammation during COPD treatment. The present study confirmed previous studies regarding the possible role of inflammation in this group of COPD patients.[7,8,13]

In this study, 87% of patients were in moderate and severe stages of COPD according to GOLD staging, and 56% had a BODE index $\geq$3. In light of the mean long interval of 17 years since SM exposure, it seems that respiratory complication of SM has a progressive nature even after exposure has ceased. This is also reflected in the BMI of patients, which decreased correlation with the duration of the disease. The mean 6MWD in our study was 327.80 $\pm$ 86.96 m. Teramoto et al.[24] established the normal reference value of 592 m in healthy Asian men. Therefore, it demonstrates a significant functional disability in this group of patients. This finding was consistent with Broekhuizen et al.[23] who reported the functional impairments of patients due to the inflammatory basis of their disease. Furthermore, a comparison of total SGRQ scores of SM patients with normal subjects' values[18] revealed a significant impairment in the quality of life for these patients. These results were consistent with that of Attaran and colleagues[25] who reported that disturbances in the quality of life for SM patients are a serious problem.

We did not find any significant differences in the hs-CRP levels between patients on inhaled corticosteroid therapy compared to those who were not. This may be due to the low compliance of patients, small sample size of subjects, or the suboptimal use of drugs. This finding is similar to that of de Torres et al.[7] who found that patients on inhaled corticosteroid therapy had lower hs-CRP levels, although this was not statistically significant.

However, we found elevated hs-CRP levels that were independent of any specific risk factors in SM patients. A large series of prospective epidemiological studies have convincingly demonstrated that CRP, when measured with high sensitivity assays, strongly and independently predicts the risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death among apparently healthy individuals.[26] Therefore, paying more attention to this matter may be necessary.

This study has several limitations. The first is the absence of other COPD patients (such as those with COPD due to smoking) as a control group. Second, our small sample size may limit the interpretation of our results.

Conclusion

The present study demonstrates that chronic lung disease in patients with respiratory complication of SM poisoning is associated with systemic inflammation and that level of inflammatory markers (hs-CRP) is correlated with the severity of airway disease. Serum hs-CRP significantly correlates with GOLD, FEV1, FEV1 percent predicted, and $PaCO_2$. In these patients, as in COPD, hs-CRP may play a significant role in disease severity and could be used as a marker of severity in addition to other clinically important prognostic factors.

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Conflict of interest

None of the authors have a conflict of interest to declare in relation to this work.

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